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Contract No. AF 30(602)2248
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and

Contract W-7401-eng-49 between the U. S. Atomic Energy Commission and the University of Rochester, administered by the Department of Radiation Biology of the School of Medicine and Dentistry.

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ATOMIC ENERGY PROJECT
P. O. BOX 287, STATION 3
ROCHESTER 20, NEW YORK

AND

ROME AIR DEVELOPMENT CENTER
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ABSTRACT

The interaction of different forms of energy that result in antagonism or modification of response in the organism is a recognized biological phenomenon. Evidence has been obtained that dogs exposed to a physiologically tolerated level (100 mw/cm^2) of pulsed microwaves (2800 Mc/sec) are less sensitive to ionizing radiation than animals not previously pretreated with microwaves. Simultaneous exposure to ionizing and microwave irradiation results in earlier granulocytic recovery than is seen after ionizing radiation alone. Protection by enhancement of recovery processes is considered. Lethality from whole and partial body ionizing radiation after exposure to microwaves is reduced depending on duration of previous microwave exposure. The results indicate the need for additional work with consideration of time-intensity factors of both forms of energy to permit the evaluation of these procedures in modification of injury or enhancement of recovery after ionizing radiation exposure.

The mechanism of radiation injury and the natural processes of recovery have eluded investigators for many years. Numerous approaches have been considered for altering both the direct and indirect effects of irradiation experimentally. None of these have developed into a practical solution to this problem. Protection by the use of chemicals is limited because these agents must be given immediately before irradiation and be present in effective concentration in the organism during irradiation.

Ellinger (11,12) has reviewed synergism and antagonism in the effects of radiationon the biologic system. The suggestion is made that two types of radiationmay act on different biologic substrates inducing physiologic mechanisms which may either supplement or oppose each other. The effects of ultraviolet radiation on unicellular organisms can be counteracted by visible light (photo reactivation) (17,18).

The ultraviolet and visible light regions lie between the ionizing and microwave bands of the electromagnetic spectrum. It could be possible that the latter two radiant energies may have some synergistic or antagonistic action on biologic systems.

The effects of whole body exposure to microwaves has not been investigated as completely as those of ionizing radiation.

Irradiation of dogs with 10.7 cm pulsed microwaves produces physiologic alterations which are not completely understood (20). Exposure to 100 mw/cm^2 up to six hours, in which rectal temperature increase is slight, results in a marked eosinopenia and lymphocytopenia. A rebound of these cellular elements is apparent in 24 or 48 hours. The decrease in lymphocytes and eosinophil levels seen after prolonged exposure to microwaves may be indicative of hypothalamic or adrenal stimulation.

An early manifestation of microwave exposure is hemodilution during the first 30 minutes of exposure and before body temperature increases (15). With prolonged exposure, hemodilution is reversed as a result of dehydration and hemoconcentration occurs. The early hemodilution is no doubt due to an influx of extravascular fluid which follows the extensive peripheral vasodilatation which has taken place (20). The long term influence of these hematologic and hemodynamic alterations on the animal is not known.

Dogs repeatedly exposed to tolerable dosages of microwaves become adapted to the procedure as indicated by the development of a lower than normal basal temperature (22). The effect of lowered body temperature on the response to ionizing radiation has been reported (10,23,25).

The lack of studies on the possible interaction between microwave and ionizing radiation in the mammalian organism has stimulated the following investigations.

METHODS

An AN/MPS-14 (2800 Mcycle/sec pulsed) radar set was used for microwave exposures. The animals were maintained in a plexiglass cage 23" long x 12" wide x 24" high, situated in an anechoic chamber 7' x 7' x 15' long. Power levels of 100 mw/cm^2 or 165 mw/cm^2 were used.

X-irradiation was from a 250 KVP Andrex industrial x-ray unit at a dose rate of 2 r/minute. The half value layer for this irradiation was 0.65 mm Al.

Five dogs received simultaneous whole body x-ray and microwave exposure; one was exposed at a time. The x-ray and microwave beams intersected at a 90° angle in the center of the cage. Eight additional dogs received identical ionizing radiation exposure without microwaves.

Another group of animals consisting of 62 normal dogs and 59 dogs that were exposed to microwaves under a variety of experimental conditions were subjected to ionizing radiation from a 1 Mev General Electric X-Ray Unit (HVL 3 mm lead). Exposures were bilateral, whole body, lower body (from the xiphoid process caudad), or to the head alone. A segmental lead shield and a plywood restraint box were used for partial body exposures (13). The dose rate was 55-58 r/minute for whole and lower body irradiation. Head irradiation was given at a rate of 590 r/minute, the energy source directed in the midsagittal plane to the cranium.

Blood was obtained from the jugular vein. Total white cell count, differential, platelet count (indirect method), and hematocrit were determined by standard laboratory procedures.

All animals were maintained on a commercial meal diet and water provided ad libitum.

RESULTS

Simultaneous X-Ray and Microwave Exposure

The mean leukocyte response plus and minus one standard error is illustrated in Figures 1, 2, and 3.

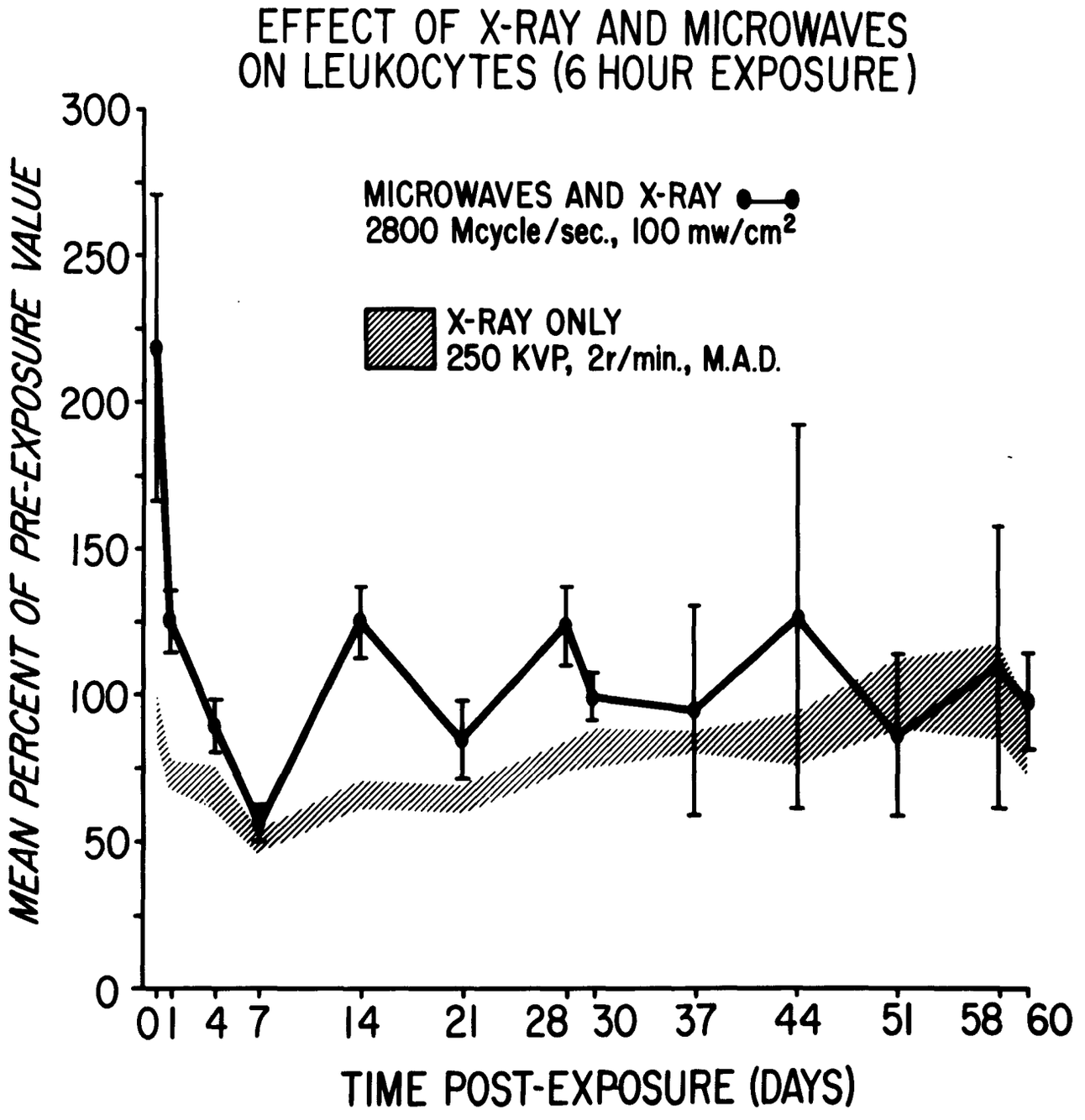
The leukocyte level was elevated (217% $\pm$ 54%)* from the pre-exposure count immediately after simultaneous microwave and x-irradiation and relatively unchanged (94% $\pm$ 7%) after x-irradiation alone (Figure 1). This was followed by a leukopenia with maximal decrease of 50% seven days after either x-ray or simultaneous exposures. Leukocytes in the dogs that received simultaneous irradiation returned to the pre-exposure level ten days after radiation In dogs that received x-irradiation only, leukocyte return to pre-exposure level occurred fifty days after treatment.

* Mean $\pm$ Standard Error of the Mean

FIGURE 1

EFFECT OF X-RAY AND MICROWAVES ON LEUKOCYTES

(6 HOUR EXPOSURE)


EFFECT OF X-RAY AND MICROWAVES
ON LEUKOCYTES (6 HOUR EXPOSURE)
MEAN PERCENT OF PRE-EXPOSURE VALUE
300
250
200
150
100
50
0
MICROWAVES AND X-RAY
2800 Mcycle/sec., 100 mw/cm²
X-RAY ONLY
250 KVP, 2r/min., M.A.D.
0 1 4 7 14 21 28 30 37 44 51 58 60
TIME POST-EXPOSURE (DAYS)

The general neutrophil response was similar to that of the total leukocytes except that the immediate post exposure increase was 384% ± 114% of the pre-exposure level (Figure 2).

At termination of x-irradiation, the lymphocyte count was 80% ± 27% of the pre-exposure value, and 40.2% ± 9% after simultaneous exposure (Figure 3). A further decrease to 60% was evident one day later in dogs exposed to x-rays alone. Lymphocyte levels in all cases were unchanged until seven days after radiation, when a progressive trend toward initial values was noted. Pre-exposure value was reached 35 days after x-irradiation and remained at this level until determinations terminated. Twenty-eight days after simultaneous exposure, lymphocyte level was 75% of pre-exposure value and remained relatively unchanged thereafter.

Rectal temperature decreased 1.43 F following x-irradiation and increased 1.13 F after simultaneous microwave and x-ray exposure (Table I).

After x-irradiation, percent body weight loss was 0.58 ± 0.28, and 6.12 ± 0.96 after microwave and x-ray exposure (Table I).

No unusual response was seen in the dogs during any of the exposures. In general, the animal was calm, occasionally changed position, or slept during the exposure. Two to three months following radiation, patchy greying of the coat resulted. Mortality did not result from either x-ray or x-ray - microwave exposure.

X-Irradiation After Microwave Exposure

The thirty-day mortality for dogs that received x-irradiation after microwaves is indicated in Table II.

Among 30 dogs with various histories of microwave exposure, the 30-day lethality was 47% after exposure to 340 r MAD. This is the LD 68/30 for normal dogs in this colony.

FIGURE 2

EFFECT OF X-RAY AND MICROWAVES ON NEUTROPHILS

(6 HOUR EXPOSURE)

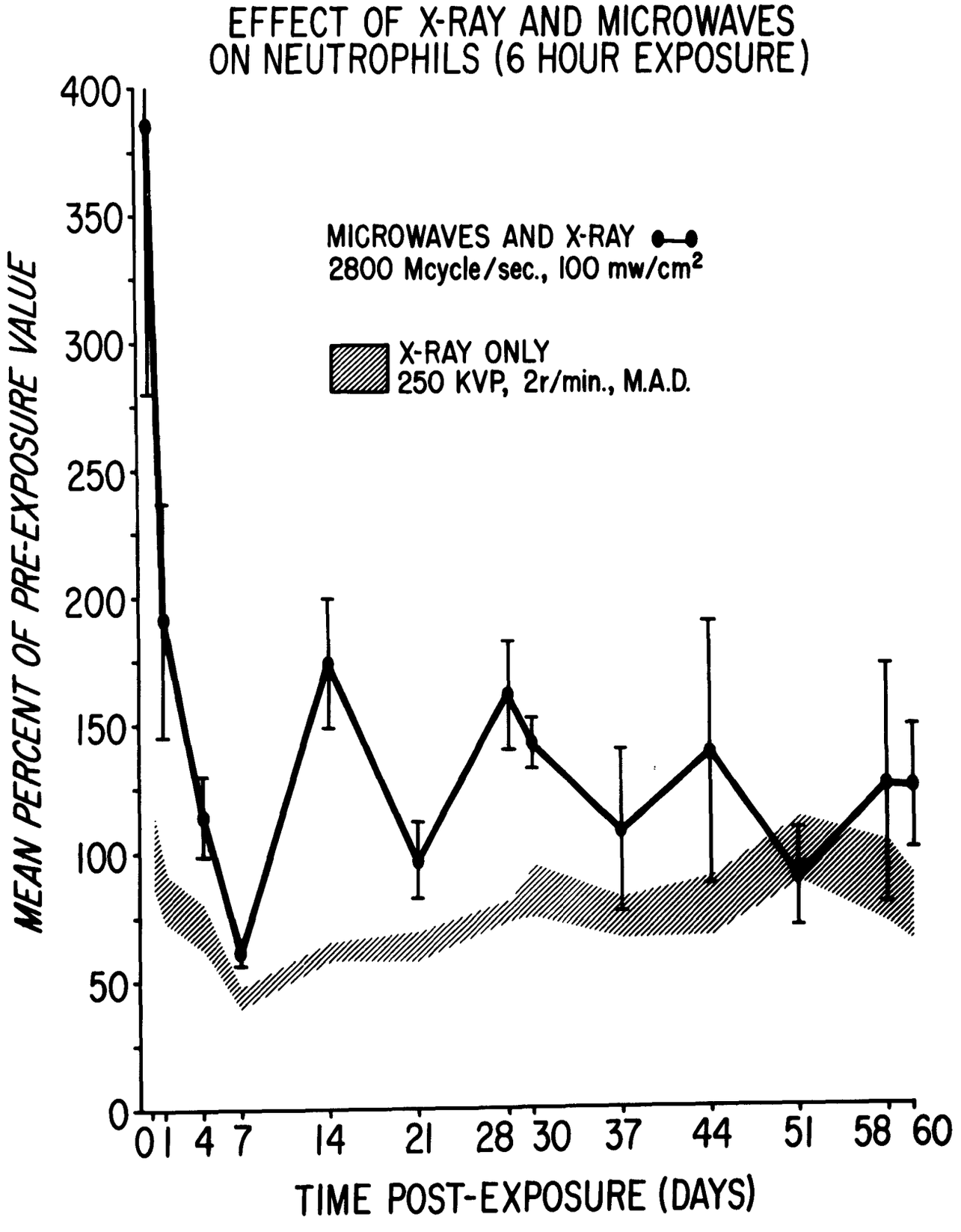

EFFECT OF X-RAY AND MICROWAVES
ON NEUTROPHILS (6 HOUR EXPOSURE)
MEAN PERCENT OF PRE-EXPOSURE VALUE
400
350
300
250
200
150
100
50
0
MICROWAVES AND X-RAY
2800 Mcycle/sec., 100 mw/cm2
X-RAY ONLY
250 KVP, 2r/min., M.A.D.
0 1 4 7 14 21 28 30 37 44 51 58 60
TIME POST-EXPOSURE (DAYS)

FIGURE 3

EFFECT OF X-RAY AND MICROWAVES ON LYMPHOCYTES

(6 HOUR EXPOSURE)

EFFECT OF X-RAY AND MICROWAVES ON LYMPHOCYTES (6 HOUR EXPOSURE)

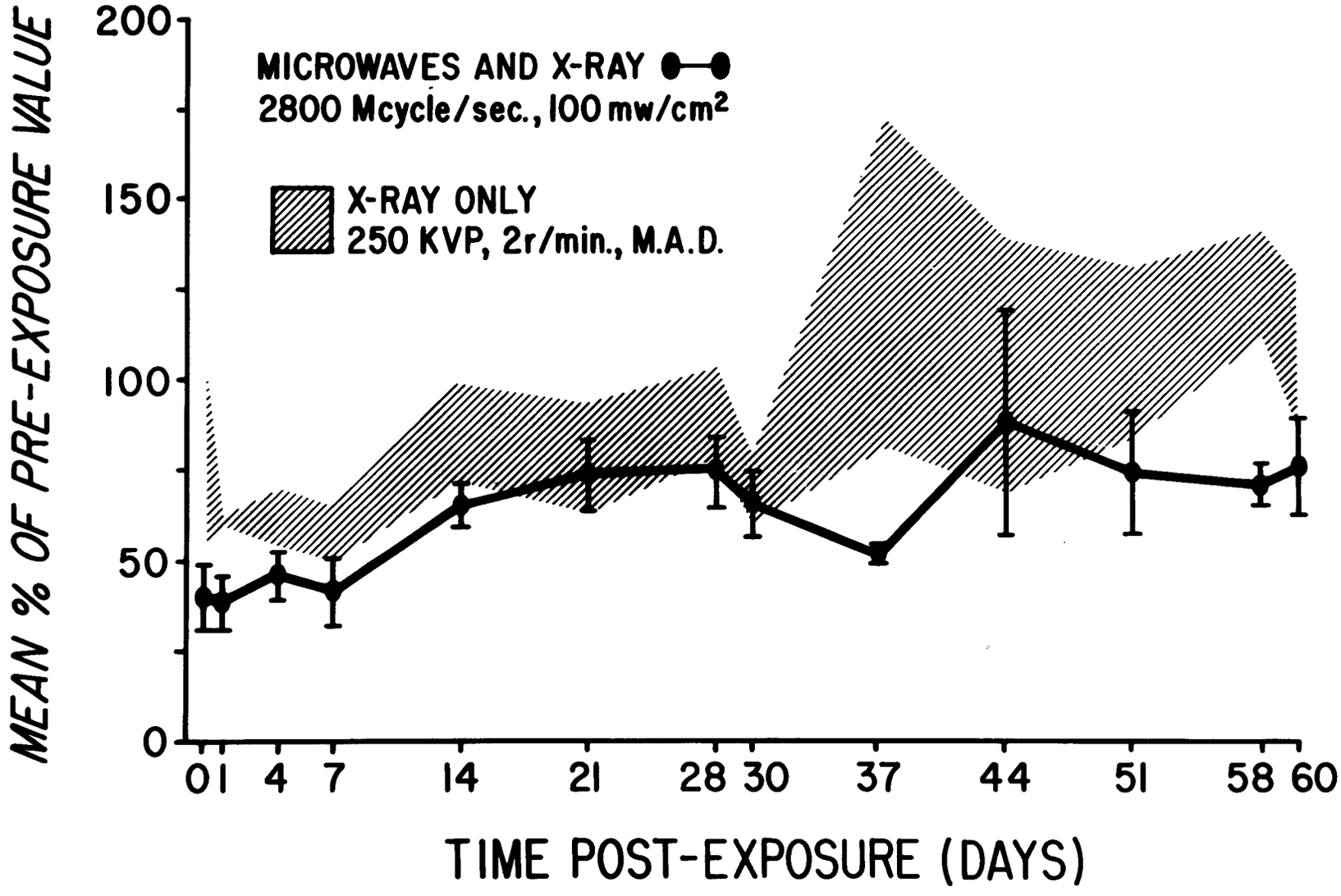


TABLE I

RESPONSE OF DOGS TO SIMULTANEOUS MICROWAVE AND X-RAY EXPOSURE[a]

Treatment	Number of Dogs	Rectal Temperature Change ° F	Percent Body Weight Loss
Control	3	0 (±0.31)[b]	1.80 (±0.36)
Microwaves	5	+2.50 (±0.54)	5.76 (±0.48)
X-ray	8	-1.43 (±0.33)	0.58 (±0.28)
Microwaves and x-ray	5	+1.13 (±0.34)	6.12 (±0.96)

[a] X-ray - 250 KVP, 2 r/min MAD; microwaves - 2800 Mcycle/sec, 100 mw/cm^2, duration of exposure - 6 hours

[b] Mean (± Standard Error of the Mean)

TABLE II

LETHALITY IN MICROWAVE TREATED DOGS EXPOSED TO 340 r MAD WHOLE BODY IONIZING RADIATION

(30-DAY LETHALITY)

2800 Mcycle/sec MICROWAVE RADIATION				IONIZING RADIATION		
Group	Exposure	Duration (Min)	Months Pre-Ionizing Radiation	Mortality		Mean* Survival Time (Days)
1	Normal (x-ray only)	-	-	23/34	67.6%	14.9 ± 0.62
2	100 mw/cm^2 (single)	180-360	5-32	1/6	16.7%	11.0
3	100 mw/cm^2 165 mw/cm^2 (multiple)	270-2730	7-30	4/10	40%	17.5 ± 1.50
4	165 mw/cm^2 (single)	120-450	4-21	6/10	60%	17.3 ± 1.02
5	100 mw/cm^2	30-90	1-15	3/4	75%	16.7 ± 1.18

* Mean (± Standard Error of the Mean)

When the dogs are grouped according to their microwave history (Table II), the influence of microwaves on lethality after ionizing radiation becomes more apparent. Dogs in Group 2 that had been exposed at 100 mw/cm^2 for three to six hours had a 17% mortality. Lethality in animals exposed to the same power level of microwaves but for a shorter time period (Group 5) was similar to dogs that had not received microwaves. If these two groups of microwave treated dogs are combined, the lethality is 40%. This lethality is significant by Chi square at $P > 0.05$, < 0.1 and indicates some decrease from the expected mortality. A similar decrease in expected lethality is seen in Group 3 where the animals received multiple exposures at both 100 mw/cm^2 and 165 mw/cm^2 during adaptation studies.

Single exposure to 165 mw/cm^2, which produced marked rectal temperature increase (4-6 F) in two to three hours, did not provide any effect toward reduction of lethality from ionizing radiation.

The influence of prolonged microwave exposure on the response to ionizing radiation is more clearly shown in Table III, which is a detailed analysis of animals in Groups 2 and 5 of Table II. Of six dogs that received single microwave exposure to 100 mw/cm^2 for 180 or more minutes, only one died after exposure to 340 r MAD x-rays (17%). Under the same conditions of x-irradiation, three of four dogs with 90 minutes or less of microwave exposure died within 30 days of x-irradiation (75%).

Irradiation of the lower portion of the body (xiphoid process caudad) with 950 r MAD resulted in the death of three of eight normal dogs within four days. Of nine dogs previously exposed to microwaves, none died. Intestinal hemorrhage was less severe in the microwave group.

Lethality from ionizing radiation to the head for periods of one to thirty days after such exposures is indicated in Table IV.

TABLE III

LETHALITY IN MICROWAVE TREATED DOGS EXPOSED

TO WHOLE BODY IONIZING RADIATION

LETHALITY IN MICROWAVE TREATED DOGS EXPOSED TO WHOLE BODY IONIZING RADIATION

	2800 Mcycle/sec - MICROWAVE RADIATION Single Exposure - 100 mw/cm^2			340 r - IONIZING RADIATION	
	Dog	Exposure Time (Min)	Months Pre-Ionizing Radiation	Survival Time (Days)	30-Day Mortality
1	T-8	30	11	16	
2	O-24	60	1	1[illegible]	
3	O-20	90	1	15	
4	4661	90	15	30	3/4--75%
1	T-1	180	9	11	
2	4614	180	13	30	
3	4380	180	26	30	
4	A-43	330	5	30	
5	A-36	360	12	30	
6	4322	360	32	30	1/6--16.7%
Normal Dogs					23/34--67.6%

TABLE IV

SURVIVAL TIME FOR DOGS THAT RECEIVED IONIZING RADIATION TO THE HEAD

Midline Air Dose (r)		No. of Dogs	Survival Time
25,000	Normal	10	22.0 (±1.37)* hours
	Microwave	10	43.1 (±6.70) hours
10,000	Normal	5	13.8 (±0.73) days
	Microwave	5	15.4 (±1.03) days
5,000	Normal	5	40.6 (±4.68) days
	Microwave	5	43.6 (±15.4) days

* Mean (± Standard Error of the Mean)

Administration of 5,000 r to the head did not result in any immediate clinically noticeable effect. Generalized desquamation of the irradiated area was evident in all survivors. One of five normal and two of five microwave pre-treated dogs died within the 30-day period.

Ten thousand r resulted in emesis twenty minutes after radiation in normal dogs and persisted for as long as five hours. Emesis did not occur in dogs pre-treated with microwaves. Three of five normal dogs and two of five microwave exposed dogs died fifteen days after ionizing radiation.

Irradiation with 25,000 r resulted in death within 24 hours in two of ten dogs with previous history of microwave exposure. Nine of ten normal dogs died in this time period. This difference in mortality is significant ($P < .05$) with students "t" test. Immediate neurological changes were seen in all dogs. There was loss of equilibrium, intermittent opisthotonus, nystagmus, and marked salivation. Neurological manifestations were less severe in microwave treated dogs. Microwave treated animals showed partial recovery from the acute effects prior to death, while in normal dogs the condition remained unchanged until death.

DISCUSSION

Search for methods of modification of ionizing radiation injury is important for many reasons. Among these are clinical radiation therapy, military and civilian defense planning, nuclear aircraft development, and most recently, space exploration. Of the various methods known, lead shielding is for most purposes limited by the weight requirement; chemical protectants are restricted by toxicity and short duration of action, requiring administration just prior to radiation.

Progenitive tissue, such as bone marrow, is the most sensitive to ionizing radiation. Histopathologic evidence indicates that in irradiated tissues, recovery processes are in progress even while degenerative processes are still developing during or immediately after irradiation (8,9).

Recovery from ionizing radiation is in part dependent on the regeneration of surviving cells. The lethal dose for partial body irradiation is higher than for whole body exposure with earlier hematopoietic recovery from the shielded areas (5,7). Current investigations indicate that earlier granulocytic recovery occurs in dogs exposed to high doses of ionizing radiation to the upper body in comparison with whole body irradiated dogs (19). This suggests that tissue damage to the exposed portion of the body stimulates the marrow in the shielded areas.

The observed modification in response with simultaneous exposure to ionizing radiation and microwaves may be due to several factors. Blair (6) has shown that a short period of relative resistance occurs for a number of hours after radiation exposure. In many species, an apparent bone marrow release of cells occurs for a short time during this early phase. It would thus follow that possible stimulation of surviving cells (particularly in marrow) can be achieved during this short critical time. In the current group of experiments, a quadrupling of granulocytes follows simultaneous exposure to x-rays and microwaves. This is in sharp contrast to the 25% increase in granulocytes seen after exposure to the same time-intensity factors of microwaves without x-rays (20). The marked increase in granulocytes seen with simultaneous x-ray and microwave exposure, coupled with the known stress effect of microwaves alone (20), could provide sufficient pool of viable cells to return the granulocytes to normal levels by the

tenth day after exposure. Previous investigation has indicated that exposure to microwaves (100 mw/cm^2) four days after ionizing radiation does not alter lethality (16). Microwave exposure immediately or shortly after x-irradiation is indicated to ascertain the role of recovery in this reaction. If the response is similar to that observed with simultaneous exposure, it can be concluded that recovery processes can be favorably stimulated during the short but critical time after ionizing radiation exposure.

The intensity of effects of ionizing radiation depends in part on the partial pressure of oxygen present in the irradiated system (3). This oxygen effect is observed in mammals, plants, and bacteria. Decreased venous oxygen tension from intravenously administered cysteamine is considered a contributing factor in the radioprotective activity of this agent (4). The hypoxic effect of microwaves has been mentioned by Hartman (14). Dogs exposed to microwaves have a reduced arterial oxygen saturation (21) and decreased Fe^{59} clearance 1/2 time (26) for some time after exposure.

The suggestive reduction in mortality due to ionizing radiation in dogs that received multiple exposures to microwaves to which they become adapted, or the single exposures at lower power levels (100 mw/cm^2), which permit longer exposure time, may be indicative of a microwave dependent effect. Repeated exposures to microwaves results in greater tolerance to this energy and is comparable to thermal adaptation (15). Protection against radiation injury provided by lower body temperature or the resistance of animals irradiated at reduced metabolic rates has been reported (10,23,25). Lower body temperature in these dogs may influence the biological expression of the primary ionizing radiation lesion or the recovery mechanism.

Since the nature of the chemical reaction which initiates cellular injury from either ionizing or microwave radiation is not known, one can only speculate as to the significance or mechanism of the biologic interaction of these two forms of energy. It is extremely difficult to differentiate and quantitate thermal and possible non-thermal effects of microwaves on a cellular level. The alignment of microscopic particles and cells under the influence of an alternating field with the induction of non-reversible effects has been cited by Schwann. Protein denaturation or alteration might be induced with the high field strength values present under certain conditions of pulsed radar operation (24). Biologic effects following the absorption of ionizing radiations are presumed to be due in part to chemical products whose presence and interactions result in intracellular damage (1). Such chemical reactions may not be possible in a cell previously or simultaneously altered by microwaves.

One should not ignore the possible significance of changes caused by the tremendous peaks of energy from the (360 cycles/sec) two microsecond pulse width in these investigations - approximately 2.6 seconds of "on time" per hour or 16 sec/6 hour exposure. Marked vibrational and rotational molecular changes could easily be produced by these bursts of energy. The biologic effect of such pulsations with variations in pulse height and width is another area requiring investigation.

Recent studies by Bach (2) indicate the possibility of molecular alteration after microwave exposure. Microwaves may alter the substrate upon which ionizing radiation exerts its effect and in this way change the response of the organism. It is possible that the effect of microwaves on the cell may inhibit electronic excitation, thereby preventing the formation of free radicals by ionizing radiation. Protection could

be provided by a reduction in radiation effects at the molecular level. By sparing specific sensitive cells from injury, a pool of selected viable cells could be provided for earlier replenishment of vital tissues.

CONCLUSION

The effectiveness or practicality of microwave exposure as a method for enhancement of recovery and/or protection against ionizing radiation can only be inferred from the data presented. The results suggest that additional work should be done to evaluate the potential of this procedure in counteracting or minimizing the effects of ionizing radiation.

Various time-intensity factors of ionizing radiation, frequency, power level, pulse height and width interrelationships for microwave irradiation as well as intervals between exposures of these two energies should be considered in such investigations.

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NOTICE

All experiments described in this report involving animal subjects were conducted according to the "Rules Regarding Animal Care," established by the American Medical Association.